TNF Polymorphisms Independently Predict Outcome in Patients with B-Cell Non-Hodgkin's Lymphoma

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Abstract

Objectives: Tumour Necrosis Factor α (TNFα) and Lymphotoxin α (LTα) have been implicated in the pathogenesis of lymphoproliferative disorders. Patients with B-cell non-Hodgkin's lymphoma (NHL) often have high serum levels of TNF which may be associated with a poor outcome. TNFα and LTα polymorphisms are known to influence expression of these cytokines and may explain the variable response to therapy.

Patients and Methods: In patients with NHL, serum levels of TNFα and LTα were measured. DNA was typed using allele specific PCR and restriction fragment length polymorphism for the –308 TNFα and +252 LTα polymorphisms and comparison was made with clinical outcome.

Results: The presence of high producing alleles was significantly associated with high serum levels of TNFα and LTα. The presence of 2 or more high producing alleles was significantly associated with more advanced disease at presentation (stage III and IV), p=0.024, a higher International Prognostic Index (IPI) score, p=0.038, failure to achieve a complete remission (CR) after 1st line therapy (88% vs 33%, p=0.01) and shorter progression free survival (PFS) (median 24 months compared with 78 months, p=0.001). Multivariate analysis confirmed that TNF high-risk haplotype (HRH) was an independent prognostic factor for PFS.

Conclusions: These results demonstrate that TNF polymorphisms are independent prognostic factors in NHL. Further study is required to further define the importance of TNF polymorphisms within different lymphoma subtypes and with different therapeutic approaches.

Keywords: B-cell Non-Hodgkin's lymphoma, survival, TNF polymorphisms

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Introduction

NHL is a heterogeneous group of disorders that display great diversity in their clinical course and response to therapy. A number of studies have assessed factors prognostic for treatment outcome and identified clinical characteristics of the disease such as age, clinical stage, and performance status, in addition to laboratory markers including serum lactate dehydogenase (LDH) and serum beta-2 microglobulin (β2m) as important. The IPI has been developed and adopted incorporates the patient's age, stage, LDH, performance status and the number of extranodal sites to define the patient risk at diagnosis[1,2]. In the research setting a number of biological markers such as the adhesion molecule CD44 [3], differentiation inhibitory factor nm23Hi [4] and the proliferation index Ki-67 [5] have emerged as important prognostic parameters. Despite this progress, there remains the need to identify further factors that can more accurately identify the biological nature of the disease and predict its behaviour, thus allowing therapeutic approaches tailored to the individual patient. Of particular interest in this respect, is the molecular characterisation of individual tumours.

TNFα and LTα (formerly known as TNFβ) are two related cytokines with overlapping activities, critical in immune and inflammatory responses. Both mediate their functions through the same receptors, TNFR1 and TNFR2, which are found on different cell types. Binding of the TNF proteins to their receptors can result in cell activation, proliferation or apoptosis, depending on a number of factors, thus playing a critical role in the development and function of normal lymphoid tissue particularly B-cells[6]. TNF gene inactivation has been found to be associated with absence of lymphoid follicles, splenic white pulp and Peyer's patches revealing a critical role in lymphoid development. Following TNF receptor

stimulation, gene expression profiling has shown upregulation of NFκβ, a gene responsible for activation of transcription factors involved in lymphomagenesis [7,8,9,10]

Many in vitro studies have demonstrated that TNF induces proliferation of malignant B-cells in a number of lymphoproliferative disorders including myeloma (MM), chronic lymphocytic leukaemia (CLL) and hairy cell leukaemia (HCL) [11]. These data have suggested that TNF may therefore be important in the pathogenesis and maintenance of the malignant clone in these disorders. In support of this, elevated levels of TNF have been found in patients with lymphoma and have been reported to be associated with a poorer outcome. Also the levels of TNF and its soluble receptors in plasma have been reported to predict the treatment outcome in a further study of patients with NHL [12,13].

TNFα and LTα release is controlled by 2 separate genes on the short arm of chromosome 6, within the class III region of the major histocompatibility complex (MHC). Many polymorphisms have been described in relation to both genes. In particular, the G to A transitions at nucleotide position –308 of the promoter area of the TNFα gene and at nucleotide +252 of the 1st intron of the LTα gene are known to be associated with altered gene expression. These polymorphisms result in TNF1 and TNF2 alleles of the TNFα gene and LT10.5 and LT5.5 alleles of the LTα gene. Higher serum levels of TNFα and LTα have been found with TNF2 and LT5.5 alleles respectively [14].

TNF gene polymorphisms have previously been studied as predisposing and prognostic factors in patients with B-cell lymphoproliferative disorders including, MM, HCL and CLL with conflicting results [11,15,16]. Warzocha, et al have previously reported that polymorphisms associated with increased TNF production were associated with a poorer outcome in patients with NHL [13].

In the current study, the aims were to: (i) study the TNF gene haplotypes that are associated with an increased serum level of the cytokine and (ii) investigate the relation of the TNFαgenotype with disease outcome in terms of the response to therapy and the progression free survival.

Subjects and Methods

TNFα and LTα serum levels were measured in 28 and 21 patients prior to starting any therapy and in 21 and 18 controls respectively, using an ELISA method (Immunotech-Marseille- France and R & D-Borsigsterasse- Germany respectively).

Pre-treatment levels of β2M were measured by ELISA (Immunotech-Marseille- France).

DNA extraction

DNA was extracted from peripheral blood mononuclear cells by the salting out method using a commercial kit (Nucleon Biosciences).

Detection of TNFα polymorphism

Allele specific PCR (ASPCR) was used to detect the G→A transition at position –308 of TNFαgene using primers P1-4 in Table 1. These were used to amplify a 184-bp fragment of the TNFα gene that includes the polymorphic site. Each sample was tested in 2 reaction tubes with either P1-P2, which detect the normal allele, or P1-P3 which detect the mutant allele. P4 is an internal control that was added to all the reaction tubes to amplify a DNA fragment of 531bp to ensure the presence of amplifiable DNA.

Table 1: *The sequence of primers used in TNFα (P1, P2, P3 and P4) and LTα P4 & P5) genotyping*

P1 5`TCT CGG TTT CTT CTC CAT CG
P2 5`ATA GGT TTT GAG GGG CAT GG
P3 5`ATA GGT TTT GAG GGG CAT GA
P4 5`GAG TCT CCG GGT CAG AAT GA
P5 5`CTC CTG CAC CTG CTG CCT GGA TC
P6 5`GAA GAG ACG TTC AGG TGG TGT CAT

90μμl of reaction mixture comprised: 6μl DNA, 200μmol/L dNTPs, 2mM MgCl2, 2U Taq DNA polymerase, 20pmol of P1 and 10pmol of P4. The mixture was then divided into 2 tubes and 10pmol of P2 and P3 were added. Reaction conditions used with the thermal cycler (Hybaid) were as follows: initial heating at 95°C for 5 minutes, 31 cycles of denaturation for 30 seconds at 95°C, annealing for 30 seconds at 60°C and extension for 30 seconds at 72°C and final extension at 72°C for 7 minutes. The DNA bands were detected using UV light after being run on a 2% agarose gel (Fig 1).

Detection of LTα polymorphisms:

Assessment of the G→A polymorphism at position +252 of the LTα gene was performed in 2 stages – initial PCR amplification followed by restriction enzyme digestion.

Initially, amplification was performed using the primers P5 and P6 (Table 1).

The reaction mixture volume was 35μl consisting of 3μl DNA, 200μmol/L dNTPs, 2mM MgCl2, 1U Taq DNA polymerase and 10pmol of each primer. Following initial denaturation at 95°C for 10 minutes,

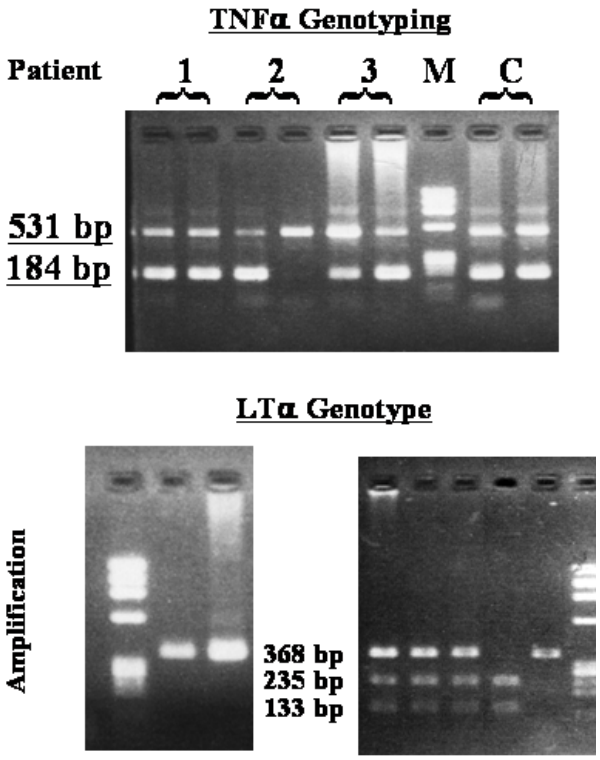


Figure 1: *Gel Electrophoresis of PCR Products*
Upper gel: *TNFα genotyping: 1, 2 and 3 represents the PCR product of 3 patients. In all patients, 1st lane represents the amplification of the normal allele and lane2 represents the amplification of the mutant allele. M = Marker and C = Control (Heterozygous). The 184 band represents the PCR product of the allele and the 531 band is an internal control. Patient 1 and 3 are heterozygous; Patient 2 is homozygous for the normal allele.* ***Lower gel:*** *LTα genotyping. Left: amplification product, 368 band. Right: PCR product after enzymatic digestion: 368 band (normal allele), 235 and 133 bands (mutant allele). Lane1 heterozygous, lane2 homozygous for the mutant allele and lane 3 is homozygous for the normal allele.*

Table 2: *Patients' Characteristics (Notice that some data are not available for all patients)*

Patients characteristics	**No**	**%**
Age:		
≤ 60	106	77
> 60	32	23
Disease Stage:		
Early (1 and 2)	66	48.2
Advanced (3 and 4)	71	51.8
IPI score:		
Low	87	77.7
High	25	22.3
Pathology:		
DLBCL	79	56.8
	34	24.4
FL	16	11.5
Others	10	7.3
Unknown		
Extranodal Sites	103	85.8
< 2	17	14.2
≥2		
BM involvement:	60	71.4
Absent	24	28.6
Present		
B Symptoms:		
Absent	83	61
Present	53	39
HB (g/dl):		
≤10	53	52
> 10	49	48
LDH:		
	66	56.9
Normal	50	43.1
> Normal		
B2microglobin (mg/ml):	33	50
≤ 3	33	50
> 3		

31 cycles of amplification were performed consisting of denaturation at 95°C for 30 seconds, annealing at 64°C for 30 seconds, and extension at 72°C for 60 seconds. The amplification products were run on a 2% agarose gel (Fig 1) and detected by UV light before proceeding to the next step.

Secondly, restriction enzyme digestion of the amplified DNA was performed using NCOI restriction enzyme. A reaction mixture consisting of 10µl of PCR product, 5U NCOI restriction enzyme, 2µl buffer and water to a final volume of 20µl was incubated for at least 4 hours at 37°C. The digested DNA bands were detected by UV light following electrophoresis on a 2% agarose gel (Fig 1).

TNFα and LTα were interpreted as an extended haplotype with patients having 0 or 1 high producing alleles of either gene considered to have a low risk haplotype (LRH) and those with 2 or more high producing alleles of either gene a HRH, as has been previously reported[14,17]. TNF extended haplotype was analysed in relation to different disease characteristics namely the clinical stage, IPI score, presence of B-symptoms, presence of bone

Table 3: *TNFα (A) and LTα (B) gene distribution and allele frequency in patients and controls*

	Allele frequency		Genotype distribution		
	TNF1	TNF2	1-1	1-2	2-2
Patients	209 (75%)	69 (25%)	79 (56.8%)	51 (36.7%)	9 (6.5%)
Controls	152 (81%)	36 (19%)	59 (62.7%)	34 (36.2%)	1 (1.1%)

A

	Allele frequency		Genotype distribution		
	LT10.5	LT5.5	10.5-10.5	10.5-5.5	5.5-5.5
Patients	199 (71.6%)	79 (28.4)	64 (46%)	71 (51%)	4 (3%)
Controls	144 (76.6%)	44 (23.4)	53 (56.4%)	38 (40.4%)	3 (3.2%)

B

marrow (BM) involvement, Hb and β2M levels. In addition, the correlation of the TNF haplotype in relation to the disease outcome in terms of the response to therapy and the progression free survival (PFS) were also analysed. In addition to TNFα/ LTαas an extended haplotype, TNFα genotype was interpreted as a marker. The correlation between the TNFαgenotype and IPI, disease stage at the time of diagnosis and the relation to the disease outcome was evaluated. Homozygosity for normal alleles was considered as low risk genotype (LRG) while the presence of one or more mutant alleles were considered as high-risk genotype (HRG). Remission status was defined using the standardised criteria of Cheson, et al [18]. The PFS was calculated from the onset of CR to relapse or to last follow up.

As this study included a heterogeneous group of patients, a subgroup of 55 patients with DLBCL who received CHOP as first line therapy were analysed separately.

Table 4: *TNF haplotype as an independent risk factor (Logistic Regression analysis):*

Variable	Significance	Relative Risk
TNF haplotype	0.002	6.5
LDH	0.22	2.1
Clinical Stage	0.17	2.3
Age	0.9	0.9
Extranodal sites	0.09	4.3

Finally TNF haplotype was studied as an independent risk factor for PFS along with age, number of extranodal sites, LDH and clinical stage.

SPSS (10th version) statistical package was used for statistical analyses. Comparison between TNF serum levels in patients and controls was done using t-test. Chi-square test was used to compare TNF haplotype in patients and controls, to study the correlation between TNF haplotype and different disease related categorical variables including, stage, IPI score, LDH levels, B2M level, Hb levels, BM involvement, number of extra-nodal sites and response to therapy. The progression- free survival was evaluated in relation to the TNF haplotype using Log-Rank analysis. The TNF haplotype was evaluated as an independent risk factor in patients with B-cell NHL using Binary Logistic Regression. Binary Logistic Regression was used to predict a dependent variable on the basis of continuous and categorical independents; to rank the importance of independents and to assess interaction effects.

Results

The study included 139 patients with B-cell NHL, 84 patients with high-grade lymphoma (including 79 with diffuse large B-cell lymphoma and 5 with other high-grade lymphomas) and 45 patients with low-grade lymphoma (including 37 with follicular lymphoma, 4 with MALT lymphoma, 4 had low grade lymphomas other than follicular lymphoma). In 10 the pathology was undefined. Ninety-four healthy controls (in whom lymphoproliferative disorders and autoimmune disease were excluded) were also studied. The median age of patients was 51 years (range 13-76 years) and of controls was 44 years

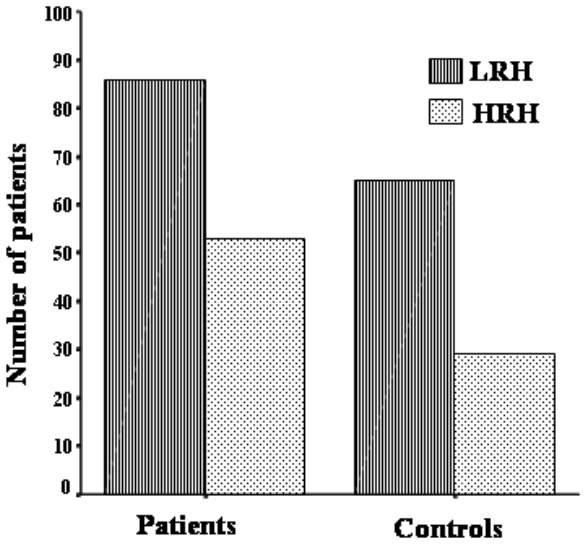


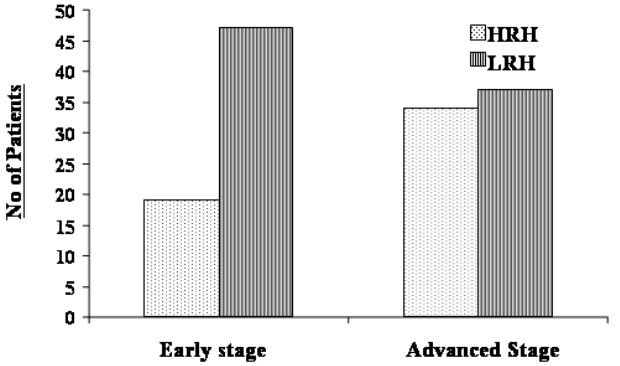
Figure 2: *Frequency of TNF Haplotypes in Patients and Controls, HRH: High Risk Haplotype, LRH: Low Risk Haplotype*

(19-65 years). The study was approved by the local ethical committee and informed consent was obtained.

The diagnosis of lymphoma was confirmed by histological examination. Staging was carried out according to standard methods. Patient characteristics are shown in Table 2.

Serum Levels of TNFα and LTα

The analysis of the serum levels of TNFα and LTα was available in 28 and 21 patients and 21 and 18 controls, respectively. The serum levels of both TNFα and LTα were significantly higher in patients compared to controls, median TNFα serum level 36pg/dl for patients and 8pg/dl for controls respectively, p= <0.001, median LTα, level for patients 13pg/dl and controls 8pg/dl respectively, p= 0.016.



There was no significant difference in the median age or incidence of TNF HRH or LRH between the groups of patients and controls in which the serum levels were evaluated.

TNF Gene Haplotype Frequency

TNF gene haplotype and allele frequency distribution in our cohort of patients are shown in Tables 3A and B and were comparable to results of other studies.

There was no significant difference in TNF gene haplotype frequency among patients and controls (Fig 2). In 139 patients, 86 (62%) had a LRH and 53 (38%) had a HRH, while in 94 controls, 65 (69%) had a LRH and 29 (31%) had HRH, (p = 0.27).

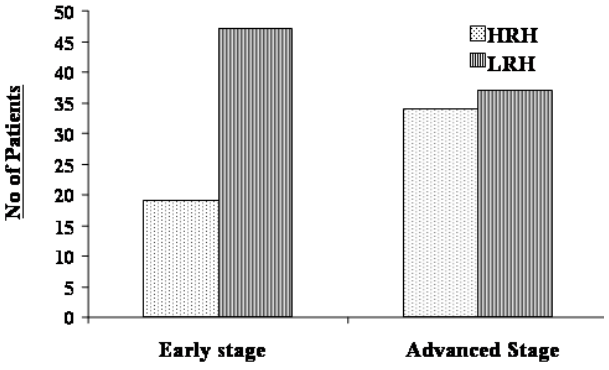


Figure 3A: *TNF Haplotype and Disease Stage at Diagnosis, HRH: High Risk Haplotype, LRH: Low Risk Haplotype*

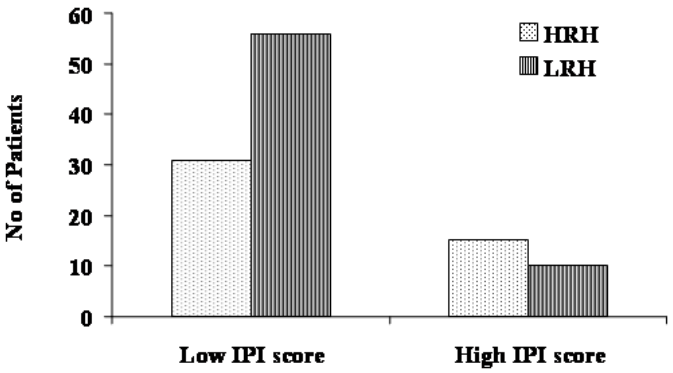


Figure 3B: *IPI Score in Relation to TNF Haplotype at Diagnosis, HRH: High Risk Haplotype, LRH: Low Risk Haplotype*

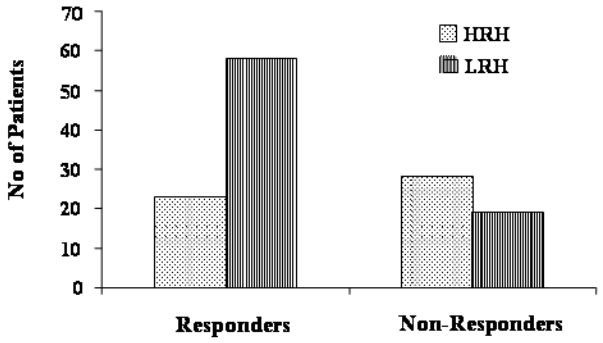


Figure 4: *TNF Haplotype and Response to Initial Therapy, HRH: High Risk Haplotype, LRH: Low Risk Haplotype*

There was no significant difference in the frequency of TNF haplotype between the high-grade and low-grade lymphoma.

Haplotype and Relation to Disease Characteristics at Presentation

In the whole patient group, patients with a HRH had a significantly more advanced stage of disease (stage III or IV) at diagnosis compared to those with LRH. Among 53 patients with HRH, 34 (64%) were found to have an advanced disease stage at time of diagnosis while within the group of 84 patients with LRH, 37 patients (44%) had an advanced disease stage (p=0.024), (Fig 3A).

The IPI score data were available in 112 patients at diagnosis and this was analysed in relation to the TNF haplotype. We found a significant correlation between the TNF haplotype and the IPI score at the time of diagnosis in our cohort. Patients with HRH were found to have significantly higher IPI scores (>2) compared to those with LRH. Among 66 patients with LRH, 84% (n= 56) were found to have low IPI score (0 –2) versus 67% of 46 patients (n=31) with HRH. This difference was statistically significant (p=0. 038). (Fig 3B)

There were insufficient patients with high IPI score to allow similar analysis in that group.

Response to Therapy

When the response to 1st line therapy was evaluated in 128 patients in relation to the TNF haplotypes, we found that the patient group with LRH achieved CR significantly more frequently than those with HRH (75%, versus 45%, p= 0.001, Fig 4)

We evaluated the response to 1st line chemotherapy in relation to TNF haplotype in the subgroups with early and advanced disease stage at the time of diagnosis. In 50 patients with early disease stage (stage I and II) CR rate was significantly higher in patients with LRH compared to those with HRH (88% and 33% respectively p=0.012). Similarly in patients with advanced disease (stage III and IV), CR rate was significantly higher in LRH patients (68.6%) than those with HRH (39.4%, p=0.028)

In patients with low IPI score (0-2) at diagnosis (n=83), CR rate was significantly higher in those with LRH (80%) compared to those with HRH (48.4%, p =0.003). In view of the small number of patients with high IPI score at diagnosis, we did not analyse the TNF haplotype in relation to disease outcome within this group.

In 79 patients with high-grade lymphoma, CR rate was significantly higher in those with LRH (76% versus 51% respectively, p=0.021). Similarly in 43 patients with low-grade lymphoma, CR rate was significantly higher in the group with LRH compared to those with HRH (76% versus 35% respectively, p=0.008).

In 75 patients with DLBCL, CR rate was significantly higher in those with LRH (74% versus 48% respectively, p=0.028). Similarly in 36 patients with FL, CR rate was significantly higher in the group with LRH compared to those with HRH (72% versus 36% respectively, p=0.04).

Patients Receiving CHOP Chemotherapy:

In a subgroup of 55 patients with DLBCL (33 with LRH and 22 with HRH) uniformly treated with CHOP chemotherapy, CR rate was significantly higher in those with LRH than those with HRH (75%, n=24 versus 43%, n= 10, p=0. 025. In a subgroup of 41 patients with low IPI score at diagnosis , response was significantly higher in those with LRH compared with the group with HRH (83% versus 47%, P=0.02).

Progression Free Survival

Assessment of PFS using Log-Rank analysis showed that PFS for the subgroup with LRH was significantly longer than those with HRH (Median 78 months versus 24 months, p=0.001).

Multivariate Analysis

We used a Logistic Regression model to evaluate the impact of TNF gene haplotype on lymphoma outcome. The Logistic Regression (Table 4) was tested by introducing the TNF gene haplotype along with patients' age, clinical stage, LDH and number of extranodal sites. TNF HRH was found to be an independent risk factor for the PFS (Relative risk 6.5, p= 0.002).

TNFα Genotype and Disease Outcome

We analysed the TNFαgenotype alone in relation to different disease characteristics and to the outcome in terms of response to therapy and the PFS.

There was no significant correlation between the TNFα allele type and either stage or IPI at the time of diagnosis. On the other hand, we found that the TNFα genotype predicted the response among 131 patients, 77% (n=57) of those with LRG achieved CR to 1st line therapy versus 23% (n=17) of those with HRG, p=0.001. In the subgroup analysis, the TNFα genotype was significantly correlated with the response in the subgroups with advanced disease stage at the time of diagnosis (n=68, p=0.01) but not within the subgroup with limited disease (n=61, p=0.07). Also, TNFαgenotype was significantly correlated with response in 83 patients with low IPI score (p=0.002). We also found that TNFα genotype was significantly correlated with PFS. Patients with LRG had PFS of 78 months versus 18 months for those with HRG (p=0.0003). Moreover when we introduced TNF genotype along with other characteristics; namely, age, clinical stage, number of extranodal sites and LDH into the multivariate analysis model, TNFα genotype was found to be an independent risk factor for the 3-year PFS (p=0.001 and relative risk 8).

Negative Associations

We did not find a significant correlation between the TNF gene haplotype and the histological type of the lymphoma, the presence or absence of B symptoms, the number of nodal or extranodal sites, the presence of BM involvement, the Hb level or the serum levels of LDH or B2m.

Discussion

Many cytokines have been found to play a role in the proliferation and differentiation of B cells including IL-2, IL-4, IL-10 and TNF. TNF has been found to function in both autocrine and paracrine fashions with previous in vitro studies demonstrating the role of TNF in supporting the growth of malignant B cells from patients with different lymphoproliferative disorders [17]. Further studies have shown significant increases in TNF production in patients with B-CLL, HCL, MM and lymphoma compared to normal controls [19,20].

It has been also recognised that higher serum levels of TNF are associated with poorer outcome in patients with NHL and CLL. Fowler et al 2001 reported that TNFα and IL-4 were higher in B CLL patients compared to controls and that both cytokines contributed to the growth of malignant B cells [17]. Ferrajoli et al 2002 reported that TNFα level was a novel prognostic factor for survival and could be used for monitoring the response in patients with CLL [21]. In NHL patients, higher levels of TNFα and its soluble receptor were found to be associated with adverse prognostic features and a poorer outcome [12,13].

As TNF production is under genetic control and certain haplotypes are associated with increased production and higher levels, we studied the TNF haplotypes in relation to the different disease characteristics at the time of diagnosis that are known to affect outcome and the relation of the TNF haplotype with disease outcome in terms of response to treatment and PFS. In this study there was no difference in TNF haplotype frequency between NHL patients and controls indicating that the presence of a HRH is not a risk factor for the development of lymphoma. These results confirmed those reported by Warzocha et al in 1998 and Fowler et al in 2000[14, 22]. In our series, however, HRH was significantly associated with more advanced disease stage and higher IPI score at the time of diagnosis compared to LRH.

We also found that a HRH was significantly associated with failure of response to 1st line therapy in all patient groups. In the subgroup of patients with low IPI scores (score 0-2) who are expected to have a good prognosis, HRH was associated with poor outcome, Thus, incorporating TNF haplotype into prognostic model may help to better deline the prognosis in subgroups that are not identified by current parameters. In addition, in the subgroup of patients who received CHOP as 1st line therapy for DLBCL, TNF haplotype was significantly correlated with the response evaluated after 6 cycles of therapy. TNF haplotype was significantly correlated with response in their patients[14]. However, their subgroup analysis for patients with diffuse large cell lymphoma showed no significant difference in response in patients with HRH and LRH. There was no analysis for the subgroup with high and low IPI score. In the current study, PFS was significantly shorter in patients with a HRH, as previously shown by Warzocha, et al [14].

We also looked at the relation of TNFα genotype alone in relation to the different disease characteristics, as it has been suggested previously that this may be as predictive as TNF haplotype. In our patient group, TNFα HRG was significantly associated with lower response to treatment also and shorter PFS but not with disease stage or IPI score at the time of diagnosis. Also there was no significant correlation between TNFα genotype and response in the subgroup of patients with limited disease. Thus, in this study, the use of TNF haplotype provide a better overall prediction of outcome than the use of TNFα genepolymorphism alone and confirmed the previous

findings of Warzocha et al that study of TNF polymorphisms in patients with lymphoma is a simple and rapid test that adds significant prognostic information to the currently available prognostic indexes.

In our study the patient group was heterogeneous in the type of lymphoma, and it is possible that TNF polymorphisms may not have the same prognostic relevance in all lymphoma subtypes. Future study of more homogenous cohorts of patients with similar lymphoma subtypes and receiving similar therapy is therefore warranted to further define the role of TNF polymorphisms in predicting outcome.

Warzocha et al have extended their studies to polymorphisms at other sites of the TNF genes including positions -376, -238, -163 of the TNF(gene, +252 of LT gene and additionally HLA DRB1. In 204 NHL patients, they found that TNF(–308A allele, null HLA DRB1*02 allele or both were associated with shorter PFS and overall survival with multivariate analysis showing these as independent prognostic factors. However, polymorphisms at other sites did not appear to be predictive of outcome [23].

Biologically, the deleterious effect of high producing TNF alleles may be explained by the autocrine and paracrine effect of TNF as a growth factor for malignant B cells [21]. Microarray studies of gene expression have shown that TNF ligand-receptor binding upregulates the gene expression of NF B. This gene is known to stimulate many transcription factors with subsequent increases in B cell proliferation. NF- B also has a role in the inhibition of apoptosis and can stimulate the transcription of TNF itself. It is however possible that TNF haplotype is linked to other genetic factors that may be significant in determining the outcome [24,25].

An increasing number of biological factors are being recognised as predictors of outcome in NHL patients. In diffuse large cell lymphoma, Bcl-2 overexpression has been shown to be associated with a poor prognosis, while Bcl-6 mutations and CD10 expression were associated with a better outcome [26].

Recently, microarray studies of gene expression have shown that refinement of lymphoma classification and more accurate prediction of the disease behaviour is possible along with improved understanding of which genes are deregulated in lymphomagenesis. Ultimately, molecular profiling of this type may allow a formal genetic assessment of each individual tumour and allow more informed tailoring of therapy on an individual basis [27,28,29,30].

This study has confirmed that TNF gene polymorphisms are an independent predictor for outcome in NHL patients. Further work is required to identify the role of TNF polymorphisms in different histological subtypes and with different treatment modalities to fully assess their role in predicting outcome. However, as more biological predictive factors are identified, consideration will need to be given to their incorporation into the currently utilised prognostic models or the development of new models which may better define responses to therapy and outcome.

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